Determination of oxygen supply and consumption

Principle: polarographic determination of oxygen partial pressure in perfusate before and after perfused HL, oxygen concentration calculated on the basis of the Henry-Dalton law

$$(1) = \frac{(O2,\ HB\text{-bound} + O2,\ phys.\ diss.)_B}{time \times muscle\ mass}$$

$$(2) = \frac{(O2,\ HB\text{-bound} + O2,\ phys.\ diss.)_A}{time \times muscle\ mass}$$

B: before perfused HL
A: after perfused HL
oxygen supply = (1)
oxygen consumption = (1) - (2)

$$\text{oxgen extraction} = \frac{\text{consumption} \times 1oo}{\text{supply}}$$

Time: -

Sample material and quantity: perfusate, o.5 ml before and after perfused HL, sites of sampling: see FIGURE ..

Results expressed in:

(1) supply, consumption or extraction at the time of sampling
(2) mean supply, mean consumption or mean extraction of each interval (= mean of start and end of interval)

supply and consumption: umol/(min x g muscle WW)

extraction: o/o

Equipment: Astrup Micro Equipment: AME1 or BMS3 MK2 and Digital Acid-base Analyzer PHM7 MK2
Radiometer A/S,
DK-24oo Copenhagen, Denmark
via
Hillerkus,
D-415o Krefeld-Bochum

2501091316

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

tuberculin syringes, polypropylene,
1 ml, no. 941oooo,
Asik Hospital Supplies,
Denmark

Procedure

according to Clark, L.C., Tr. Am. Soc. for Art. Int. Organs 2: 41 (1956)

Calculations:

(1) O2, phys. dissolved ((O2)P):

$$(O2)P = pO2 \times \frac{o.o24 \times 1ooo}{76o}$$

pO2: oxygen partial pressure (mm Hg)

(2) O2, HB-bound ((O2)HB):

$$(O2)HB = \frac{sO2corr. \times 1.39 \times (HB)}{1oo}$$

sO2corr.: oxygen saturation corrected for temperature and pH according to Severinghaus, W. in: Altman, P.L., Dittmer, D.S. (Eds.): Blood and other body fluids, Washington DC: Fed. Amer. Soc. Expl. Biol. 1961 (see FIGURES . to .)

sO2corr. pO2/(pH corr. factor x temperature corr. factor)

Hb: hemoglobin concentration (g/l)

(3) oxygen supply (OS):

$$OS = \frac{((O2)Hb + (O2)p) \times FR}{22.4 \times BW/1o}$$

BW = body weight (g)

FR = flow rate (ml/min)

(4) oxygen consumption (OC):

$$OC = (OS)_B - (OS)_A$$

2501091317

Scientific version: 5.Feb.8o
Text version: 15.Jun.81

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Preparation of perfusate supernatant

Principle:	separation of perfusate supernatant from erythrocytes by centrifugation
Time	
Sampling:	-
Determination:	within 2 min after sampling
Sample material and quantity:	perfusate, 1.o ml
Results expressed in:	-
Equipment:	centrifuge: type "Eppendorf", no. 5412, micro vials: type "Eppendorf", no. 381o, polypropylene, Netheler und Hinz, D-2ooo Hamburg 63
Chemicals and reagents:	-
Procedure	
Centrifugation:	9.76x1oE4 m/s2 (= 995o x g) for 2 min at RT, transfer of perfusate supernatant to fresh micro vial
Storage:	at o to 4 degrees centigrade
Scientific version:	5.Feb.8o
Text version:	24.Sep.81

2501091318

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Determination of phagocytic index

Principle:	impedance change of counting (Coulter principle) of fungal spores phagocytized (attached or engulfed)
Time	
Sampling:	on day ...
Determination:	within 4 h after start of harvest of free lung cells
Sample material and quantity:	free lung cells, o.5x1oE6 macrophages per incubation dish
Results expressed in:	spores/macrophages
Equipment:	incubation dishes: petri dishes, no. 5221, 35 mm x 1o mm, Lux Scientific Corporation, California, USA
	incubator: W.C. Heraeus GmbH, D-325o Hanau
	sonifier: B-15, titanium microtip, Branson Sonic Power Company, Danbury, Connecticut, USA
	particle counter: Coulter Counter model ZF, Coulter Electronics Limited, D-415o Krefeld
	centrifuge: model J6, rotor: JS-4.2, Beckmann Instruments GmbH, D-8ooo München 4o
	microscope: 4oo-fold magnification, standard 14, objective: PL 4o/o.65, Carl Zeiss, D-7o82 Oberkochen

2501091319

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Chemicals and reagents:

cell suspension medium:
medium 199 (modified), with Earle's salts, without sodium bicarbonate, without glutamine, with 2o mmol/l Hepes buffer and addition of Penicillin and Streptomycin,
no. 12-2o4-49,
fetal bovine serum, filtersterilized, mycoplasma and virus screened,
no. 29-1o1-49 (4-55D),
Flow Laboratories GmbH,
D-53o9 Meckenheim

Isoton 2,
Zaponin,
Coulter Electronics Limited,
Hertfordshire, United Kingdom

spores of the basidiomycete Geastrum triplex Jungh, diameter: approx. 5 um,
University of Gothenburg, Sweden

phosphate buffered saline (PBS), Instamed PBS, calcium-and magnesium-free, no. 182-1, pH 7.2,
Seromed GmbH,
D-8ooo München 71

Triton X-1oo, no. 37238,
penicillin, potassium salt, no. 31749,
streptomycin sulfate, no. 355oo,
Serva Feinbiochemica GmbH and Co.,
D-69oo Heidelberg 1

Procedure

Preparation of fungal spores:

spores suspended and boiled in o.o3 g/l Zaponin for 5 min, suspension centrifuged at 3.2x1oE4 m/s2 (= 33oo x g) for 1o min. After 5 subsequent washes of the spores in bidistilled water, spores resuspended to a concentration of 1.4 to 1.7x1o E7/ml bidistilled water and stored at minus 3o degrees centigrade. Prior to addition to free lung cells, thawed spores centrifuged at 3.2x1oE4 m/s2 (= 33oo x g) and resuspended in cell suspension medium with 1oo ml/l fetal bovine serum to a concentration of 1.4 to 1.7x1oE7/ml

2501091320

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Phagocytosis assay:	a defined number of free lung cells in medium 199 mixed and incubated in a petri dish for 9o min at 37 degrees centigrade (control: 4 degrees centigrade) with 1.5x1oE7 pretreated fungal spores in the presence of 5o ml/l fetal bovine serum during incubation and phagocytosis of spores most phagocytic cells adhered to the bottom of the dish and were retained there during the subsequent 2 washes of the cells with PBS. Retained cells destroyed by sonication in the presence of the detergent Triton X-1oo, spores (adherent to or engulfed by these cells) released into supernatant and counted with particle counter.
Scientific version:	19.Sep.8o
Text version:	3o.Sep.81

25010913 21

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Determination of phagoluminescence

Principle:	determination of luminescence during the incubation of suspended free lung cells with Zymosan particles in the presence of luminol
Time	
Sampling:	on day ...
Determination:	within .. hours after start of harvest of free lung cells
Sample material and quantity:	free lung cells, 5x1oE5 macrophages/ determination
Results expressed in:	(1) log cpm/1oE3 macrophages (2) cpm/1oE3 macrophages
Equipment:	pH-meter: PW 94o9, glass electrode: CA/14o2 Philips GmbH, D-35oo Kassel
	osmometer: no. 77o58, KG Wissenschaftlicher Gerätebau Dr. Knauer und Co. GmbH, D-1ooo Berlin 37
	centrifuges: model J6, rotor: JS-4.2, Beckmann Instruments GmbH, D-8ooo München 4o
	model 5412, Netheler und Hinz, D-2ooo Hamburg 63
	scintillation counter: Mark 3 model 688o,
	scintillation vials: polypropylene, no. 714, W. Zinsser, D-6ooo Frankfurt/Main

2501091322

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

microscope: standard 14,
objective: PL 4o/o.65,
Carl Zeiss,
D-7o82 Oberkochen

Coulter mixer C5,
Coulter Electronics Limited,
D-415o Krefeld

Chemicals and reagents:

cell suspension medium:
Eagle's essential medium, according to Eagle, H., Science 13o: 342 (1959), 1o-fold concentrate, no. 14-33o-54,

fetal bovine serum, filter-sterilized, mycoplasm and virus screened, no. 29-1o1-49 (4-55D),
Flow Laboratories GmbH,
D-53o9 Meckenheim

glutamine, no. 22942,
Hepes, no. 25245,
penicillin, no. 31749
streptomycin, no. 355oo,
Serva Feinbiochemica GmbH und Co.,
D-69oo Heidelberg 1

sodium bicarbonate, no. 6329,
E. Merck,
D-61oo Darmstadt 1

Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), no. A 8511,

Zymosan A from Saccharomyces cerevisiae, no. Z 425o,
Sigma Chemie GmbH,
D-8021 Taufkirchen

Procedure

Determination:

free lung cells, luminol and Zymosan particles suspended or dissolved in cell suspension medium at RT. Background luminescence of a known number of free lung cellsin cell suspension medium (1 ml/5x1oE5 macrophages) with luminol determined with the photomultiplier of a scintillation counter system 1o minutes prior to the addition of Zymosan particles. Luminescence of the incubation mixture determined at approx. 1o min intervals during the subsequent 18o min.

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Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Calculation:	substraction of background luminescence from luminescence maximum. Calculation of luminescence for 1x1oE6 macrophages.
Scientific version:	23.Sep.8o
Text version:	24.Sep.81

2501091324

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Determination of potassium concentration

Principle:	flame photometric determination of the intensity of the characteristic potassium emission in relation to the emission of an internal lithium standard
Time	
Sampling:	-
Determination:	within ... .. after sampling
Sample material and quantity:	(1) serum, 24 ul (2) urine, 24 ul (3) perfusate supernatant, 24 ul
Results expressed in:	mmol/l
Equipment:	KLiNa-flame photometer, no. 65271o, Beckman Instruments GmbH, D-8ooo München 4o
Chemicals and reagents:	internal lithium-standard: 15 mmol/l, no. 663229 sodium/potassium standard: sodium: 14o mmol/l, potassium: 5 mmol/l, no. 663223, Beckman Instruments GmbH, D-8ooo München 4o
Procedure:	according to manufacturer's recommendations storage until determination: 4 degrees centigrade
Scientific version:	6.Mar.79
Text version:	19.Aug.81

2501091325

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Preparation of protein for SDS polyacrylamide electrophoresis

Principle:	dissociation of protein subunits with a sulfhydryl compound (beta -mercaptoethanol) and denaturation as well as surface coating with a negatively charged detergent (SDS)
Time	
Sampling:	on day ..
Determination:	within ... days after sampling
Sample material and quantity:	tissue homogenate or 2.9x1oE5 m/s2 (= 3oooo x g) pellet, 1 mg protein
Results expressed in:	-
Equipment:	micro vials: no. 381o, thermostat: no. 34o1, Netheler und Hinz GmbH, D-2ooo Hamburg 63 whirlmix: no. 34526, Cenco Instrumenten, Breda, The Netherlands analytical balance: model 2oo1 MP, Sartorius GmbH, D-34oo Göttingen pH meter: no. PW 94o9, glass electrode: no. CA/14o2, Philips GmbH, D-35oo Kassel
Chemicals and reagents:	disodium hydrogenphosphate 2-hydrate, no. 658o, sodium dihydrogenphosphate 1-hydrate, no. 6346, beta-mercaptoethanol, no. 8o574o, glycerine, 87o ml/l, no. 4o94, bromophenol blue, no. 8122, E. Merck, D-61oo Darmstadt 1 sodium dodecyl sulfate, no. 2o76o, Serva Feinbiochemica GmbH, D-69oo Heidelberg 1

2501091326

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

iodoacetamide, no. I 6125,
Sigma Chemie GmbH,
D-8o21 Taufkirchen

Procedure:

protein incubated for 5 min at 1oo degrees centigrade in SDS phosphate buffer, pH 7.o, containing beta-mercaptoethanol. Thereafter addition of bromophenol blue as tracking dye and glycerine to increase sample density. Further incubation with iodoacetamide at 37 degrees centigrade for 15 min to prevent aggregation of subunits.

final concentrations of components in incubation mixture:

protein	1 g/l
SDS	6.5 g/l
phosphate buffer, pH 7.o	47 mmol/l
beta-mercaptoethanol	125 ml/l
bromophenol blue	o.15 g/l
glycerine	25o ml/l
iodoacetamide	62.5 mmol/l

storage:

minus 2o degrees centigrade, stability unlimited

Scientific version: 7.Jan.81
Text version: 9.Oct.81

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Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Determination of protein concentration (Bio-Rad method)

Principle:	photometric determination of a complex formed between proteins and Coomassie Brillant Blue G-25o. The absorbance maximum for an acidic solution of the dye shifts from 465 nm to 495 nm when binding to protein occurs.
Time	
Sampling:	on day ..
Determination:	within ... .. after sampling
Sample material and quantity:	(1) purified nucleic acid fractions (2) free lung cell lysate, 1oo ul
Results expressed in:	g/l
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen
Chemicals and reagents:	Bio-Rad Protein Kit, no. 5oo-ooo6, standard protein (bovine gamma globulin), no. 5oo-ooo5, Bio-Rad Laboratories, D-8ooo München 5o
Procedure:	according to Bradford, M. M., Anal. Biochem. 72: 248 (1976), photometric determination: wavelength: 595 nm standard curve: see FIGURE ..
Scientific version:	17.Mar.8o
Text version:	24.Sep.81

2501091328

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Determination of protein concentration (Biuret method)

Principle:	photometric determination of a dye complex formed between peptide bonds and the Biuret reagent
Time	
Sampling:	on day ...
Determination:	within ... .. after sampling
Sample material and quantity:	(1) serum, 1o ul (2) S9 fraction, 1o ul (3) postmicrosomal supernatant, 1o ul (4) phenol-extracted protein, 1o ul (5) microsomal pellet, 1o ul
Results expressed in:	g/l
Equipment:	ABA-1oo Bichromatic Analyzer, Abbott Laboratories, Diagnostics Division, So. Pasadena, California 91o3o, USA
Chemicals and reagents:	test set Total protein (Biuret method), no. 124281, standard protein: Precimat, no. 1256o1, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	according to Weichselbaum, T. E., Amer. J. Clin. Path. 16: 4o (1946), adapted by INBIFO to the bichromatic analyzer.

final concentrations of components in assay mixture:

sodium hydroxide	o.1 mol/l
potassium sodium tartrate	15.7 mmol/l
potassium jodide	14.7 mmol/l
copper sulfate	5.9 mmol/l

2501091329

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

photometric determination:

wavelength 1: 55o nm
wavelength 2: 65o nm

each sample determined in duplicates

standard curve: see FIGURE ...

Scientific version: 28.Mar.79
Text version: 9.Sep.81

2501091330

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Determination of protein concentration (Lowry-method)

Principle:	complex formation of protein with copper ions in alkaline solution and reduction of the phosphomolybdic-phosphotungstic reagent by the copper treated protein
Time	
Sampling:	on day ...
Determination:	within ... .. after sampling
Sample material and quantity:	(1) epidermal sediment, 5o to 1oo ul (2) epidermal microsomal pellet, 3o to 5o ul (3) epidermal supernatant, 1o to 5o ul
Results expressed in:	g/l
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen test tubes: no. 611-53, Sovirel, Le Vallois Perret, France pipettes: type "Eppendorf Varipette", no. 471o, Netheler und Hinz, D-2ooo Hamburg 63
Chemicals and reagents	
Chemicals:	coppersulfate, no. 2791, sodium-potassium tartrate 4-hydrate, no. 8o85, sodium hydroxide, no. 9137, sodium carbonate 1o-hydrate, no. 63, Folin-Ciocalteu-Phenol reagent, no. 9oo1, E. Merck, D-61oo Darmstadt 1 standard protein: Precimat, no., 1256o1, Boehringer Mannheim GmbH, D-68oo Mannheim 31

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Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Reagents:

(1) 2o g/l sodium carbonate 1o-hydrate in o.1 mol/l NaOH
(2) 27 g/l sodium-potassium tartrate 4-hydrate in bidistilled water
(3) 1o g/l copper sulfate in bidistilled water
(4) 1o g/l SDS in o.1 mol/l NaOH
(5) alkaline copper reagent (store not more than 12 hours):
1oo ml solution (1)
1 ml solution (2)
1 ml solution (3)
(6) diluted Folin-Ciocalteu-Phenol reagent:
1 plus 1 with bidistilled water (prepared just prior to use)
(7) 5o to 1oo ul epidermal sediment in 145o and 14oo ul solution (4), respectively,
incubation by 75 degrees centigrade for 15 min
(8) protein standard:
1 mg standard protein per ml solution (4)

Procedure:

in the order given the following volumes of solutions are pipetted into glass test tubes:

(1) standard protein solution (1o, 2o, 4o, and 8o ul)
or
5o to 1oo ul of SDS-diluted epidermal protein (7)
or
3o to 1oo ul epidermal microsomal pellet
or
1o to 1oo epidermal supernatant, filled to 1oo ul with solution (4)
(2) 15o ul bidistilled water
(3) 25o ul 1 mol/l NaOH
(4) 2.5 ml alkaline copper reagent (5)

After 1o minutes at Rt, o.25 ml diluted Folin-Ciocalteu-Phenol reagent added and mixed at once.

Mixtures left to stand for at least 3o minutes at RT.

photometric determination:

wavelength: 72o nm

standard curve: see Figure ...

2501091332

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

References: Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., J. Biol. Chem. 193 : 165 (1951)

Scientific version: 28.Sep.81
Text version: 12.Oct.81

2501091333

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Determination of pyruvate concentration

Principle:	photometric determination of NADH oxidation during formation of L-Lactate from pyruvate in the presence of lactate dehydrogenase (EC 1.1.1.27)
Time	
Sampling:	-
Determination:	within ... . after sampling
Sample material and quantity:	(1) perfusate, deproteinized, 1 ml (2) tissue extract, deproteinized, 1 ml
Results expressed in:	(1) umol/l or mmol/l (2) nmol/l
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen
Chemicals and reagents:	test set Pyruvate, no. 124982, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	acc. to Czok, R. and Lamprecht, N., in: Bergmeyer, H.U. (ed.): Methoden der enzymatischen Analyse, Vol. 2, Verlag Chemie Weinheim, 3rd ed., 1974, pp. 1491 photometric determination: wavelength: 34o nm
Scientific version:	1o.Apr.8o
Text version:	28.Sep.81

2501091334

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Determination of radioactivity (liquid scintillation method)

Principle:	determination of the light emission in mixtures of the radioactive sample with scintillation cocktail
Time:	within ... .. after .....
Sample material and quantity:	(1) lung homogenate, 1o ul (2) fractionated cellular macromolecules, 1o ul (3) urine, 1 ml (4) carbon dioxide absorber, 2oo ul
Results expressed in:	dpm (blank already substracted)
Equipment:	liquid scintillation counter: Mark 3 model 68oo, W. Zinsser, D-6ooo Franfurt/Main 5o counting vials: polyethylene, with screw caps, no. 3o714o1, W. Zinsser, D-6ooo Frankfurt/Main 5o
Chemicals and reagents:	scintillation cocktail: Insta gel, no. 6o13oo9 or permafluor 5, no. 6o13129, Packard Instrument Company, D-6ooo Frankfurt/Main
Procedure:	sample mixed with 1o ml scintillation cocktail and mixture equilibrated to counting temperature (approx. 9 degrees centigrade) in the dark automatic quench correction with experimentally determined correction standards counting time: variable program: 1 (3H) or 2 (14C) cumulative counting (4oooo cpm, 2 x sigma: 1 o/o)
Scientific version:	1.Feb.81
Text version:	28.Jan.81

2501091335

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Radioimmunoassay determination of cotinine

Principle:	binding of antigen (cotinine) to specific antibody (anti-cotinine), one of them being attached to a solid substratum, the other one being labeled. Competition of the test sample antigen for the antibody binding sites quantification of unknown cotinine concentrations by comparison with a standard curve obtained with known concentrations of cotinine
Time:	-
Sample material and quantity:	plasma or test compound solution
Results expressed in:	ng/ml
Equipment:	liquid scintillation counter: Mark 3 model 6800, W. Zinsser, D-6000 Frankfurt/Main PVC microtiter plates, immulon, M 124 A, Cooke via Dynatech, D-7310 Plochingen
Chemicals and reagents:	125I-N-(p-hydrox phenetyl)-trans-cotinine carboxamide, Institut für Biopharmazeutische Mikroanalytik, Dr. G. Neurath, D-2000 Hamburg 56 human serum albumin, essentially globulin free, no. A 8763, Sigma Chemie GmbH, D-8021 Taufkirchen 125I, labeled by New England Nuclear, D-6072 Dreieich anti-cotinine antiserum (rabbit), monoclonal anti-cotinine antibodies, INBIFO, D-5000 Köln 90

2501091336

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

goat anti-rabbit IgG, no. 412o,
goat anti-rabbit IgG peroxidase-conjugated, no. 64oo,
goat anti-rabbit IgG alkaline phosphatase-conjugated, no. 65oo,
goat anti-rabbit IgG biotin-conjugated, no. 66oo,
goat anti-mouse IgG, no. 415o,
goat anti-mouse IgG peroxidase-conjugated, no. 645o,
goat anti-mouse IgG alkaline phosphatase-conjugated, no. 655o,
goat anti-mouse IgG biotin-conjugated, no. 665o,
avidin, no. BA-ooo,
biotin peroxidase-conjugated, no. BA-1o8,
biotin alkaline phosphatase-conjugated, no. BA-1o9
Medac,
D-2ooo Hamburg 36

Tween 2o (polyethylene sorbitan monolaurate), no. P 1379,
Sigma Chemie GmbH,
D-8o21 Taufkirchen

protein A 125I together with Bolton and Hunter reagent (1.1 GBq/mg), no. IM 112,
Amersham Buchler,
D-33oo Braunschweig

Procedure: according to Voller et al. (1979),

Alternative (A):

(1) coupling of immunogen to solid surface by incubating of 2oo ul of immunogen solution in PBS at 4 degrees centigrade overnight. Removal of the fluid, washing of the plates 3 times in PBS.

(2) incubation of the wells with PBS containing 1 o/o gelatin for 1 h at RT. Washing of the plates 4 to 6 times with PBS.

drying and storage of the plates in dry atmosphere until use

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Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

(3) addition of reference anti-serum together with test sample and 1 o/o HSA, running of controls receiving known amounts of antigen instead of test sample, or non-immune serum or uncoated plates, for 2 to 24 h (to be determined). Washing of the plates 4 to 6 times with PBS

(4) addition of labeled 2nd anti-body directed against rabbit gamma globulin (in case of rabbit antiserum used) or mouse gamma globulin (in case of mouse hybridoma antibodies used)

labeled second antibodies (alternatives):

(4.1) goat anti-rabbit or goat anti-mouse gamma globulin serum, labeled with 125I

(4.2) the same antisera labeled with 125I protein A

(4.3) the same antisera coupled with alkaline phosphatase

(4.4) the same antisera coupled with horse-radish peroxidase

(4.5) the same antisera coupled with biotin, added avidin, added enzyme coupled biotin (enzyme alkaline phosphatase or horse-radish peroxidase)

incubation for 2 to 24 h, washing 4 to 6 times in PBS

(5) for (4.1 and 4.2): cutting out of single wells and counting of radioactivity in a gamma-counter

2501091338

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

for (4.3 to 4.5): addition of the appropriate substrates and buffers for enzyme reaction, incubation for 3o min, reading of the color reaction visually or with photometer (peroxidase reaction at 492 nm, alkaline phosphatase at 4o5 nm)

Alternative (B): according to Engvall (198o),

(1) coupling of antibodies to solid surface. Incubation of 2oo ul of antiserum/well in microtiter plates overnight. Removal of the fluid, washing of the plates three times. Drying and storage of the plates in dry atmosphere until use

(2) incubation of the coated plates with labeled antigen, sample of unlabeled antigen, together with 1 o/o HSA, in PBS for 4 h. Removal of the fluid, washing of plates, running of controls each lacking one of the above steps or additives

(3) determination of radioactivity bound to plates

Scientific version: 8.Jan.81
Text version: 8.Oct.81

2501091339

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Radioimmunoassay determination of nicotine

Principle:	binding of antigen (nicotine) to specific antibody (anti-nicotine), one of them being attached to a solid substratum, the other one being labeled. Competition of the test sample antigen for the antibody binding sites, quantification of unknown nicotine concentrations by comparison with a standard curve obtained with known concentrations of nicotine
Time:	-
Sample material and quantity:	plasma, or test compound solution o.1 ml
Results expressed in:	ng/ml
Equipment:	liquid scintillation counter: Mark 3 model 68oo, W. Zinsser, D-6ooo Frankfurt/Main PVC microtiter plates, Cooke via Dynatech, D-731o Plochingen
Chemicals and reagents:	G-3H-nicotine d-bitartrate, no. TRA.428, Amersham Buchler, D-33oo Braunschweig or nicotine (pyrrolidine-2 14C), 1.48 to 2.22 GBq/mmol (4o to 6o mCi/mmol) dissolved in benzene, Mr 123.1, no. NEC-689, New England Nuclear, D-6o72 Dreieich

2501091340

(a) general background information on RIA: Skelly et al. (1973)

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

anti-nicotine antiserum (rabbit, mouse), monoclonal anti-nicotine antibodies (mouse),
INBIFO,
D-5ooo Köln 9o

goat anti-rabbit IgG, no. 412o,
goat anti-rabbit IgG peroxidase-conjugated, no. 64oo,
goat anti-rabbit IgG alkaline phosphatase-conjugated, no. 65oo,
goat anti-rabbit IgG biotin-conjugated, no. 66oo,
goat anti-mouse IgG, no. 415o,
goat anti-mouse IgG peroxidase-conjugated, no. 645o,
goat anti-mouse IgG alkaline phosphatase-conjugated, no. 655o,
goat anti-mouse IgG biotin-conjugated, no. 665o,
avidin, no. BA-ooo,
biotin peroxidase-conjugated, no. BA-1o8,
biotin alkaline phosphatase-conjugated, no. BA-1o9
Medac,
D-2ooo Hamburg 36

Tween 2o (polyethylene sorbitan monolaurate), no. P 1379,
Sigma Chemie GmbH,
D-8o21 Taufkirchen

protein A 125I together with Bolton and Hunter reagent (1.1 GBq/mg), no. IM 112,
Amersham Buchler,
D-33oo Braunschweig

Procedure: according to Voller et al. (1979), see FIGURE . (2, Alternative A)

Alternative (A):

(1) coupling of immunogen to solid surface by incubating of 2oo ul of immunogen in PBS at 4 degrees centigrade overnight. Removal of the fluid, washing of the plates 3 times in PBS

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(2) incubation of the wells with PBS containing 1 o/o gelatin for 1 h at RT. Washing of the plates 4 to 6 times with PBS.

drying and storage of the plates in dry atmosphere until use

(3) addition of reference antiserum together with test sample and 1 o/o HSA, running of controls receiving known amounts of antigen instead of test sample, or non-immune serum or uncoated plates, for 2 to 24 h (to be determined). Washing of the plates 4 to 6 times with PBS

(4) addition of labeled second antibody directed against rabbit gamma globulin (in case of rabbit antiserum used) or mouse gamma globulin (in case of mouse hybridoma antibodies used)

labeled second antibodies (alternatives):

(4.1) goat anti-rabbit or goat anti-mouse gamma globulin serum, labeled with 125I

(4.2) the same antisera labeled with 125I protein A

(4.3) the same antisera coupled with alkaline phosphatase

(4.4) the same antisera coupled with horse-radish peroxidase

(4.5) the same antisera coupled with biotin, added avidin, added enzyme coupled biotin (enzyme alkaline phosphatase or horse-radish peroxidase)

incubation for 2 to 24 h, washing 4 to 6 times in PBS

(5) for (4.1 and 4.2): cutting out of single wells and counting of radioactivity in a gamma-counter

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for (4.3 to 4.5): addition of the appropriate substrates and buffers for enzyme reaction, incubation for 3o min, reading of the color reaction visually or with photometer (peroxidase reaction at 492 nm, alkaline phosphatase at 4o5 nm)

Alternative (B): according to Engvall (198o),

(1) coupling of antibodies to solid surface. Incubation of 2oo ul of antiserum/well in microtiter plates overnight. Removal of the fluid, washing of the plates three times. Drying and storage of the plates in dry atmosphere until use

(2) incubation of the coated plates with labeled immunogen, sample of unlabeled immunogen, together with 1 o/o HSA, in PBS for 4 h. Removal of the fluid, washing of plates, running of controls each lacking one of the above steps or additives

(3) determination of radioactivity bound to plates

Scientific version: 9.Jan.81
Text version: 8.Oct.81

2501091343

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Determination of RNA

Principle:	photometric determination of the colored complex between orcinol and the sugar of RNA
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	purified nucleic acid and fraction, approx. 1oo ul
Results expressed in:	ug
Equipment:	spectrophotometer: model 555, Perkin Elmer GmbH, D-777o Überlingen shaking water bath: no. 3147, Köttermann, D-3165 Hänigsen
Chemicals and reagents:	orcinol, no. o 1875, Sigma Chemie GmbH, D-8o21 Taufkirchen RNAse, yeast, no. 1o9126, Ribonucleic acid, no. 1o9215, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	according to Schneider, W. C., in: Colowick, S. P., Kaplan N.O. (Eds.): Methods in Enzymology, Vol. 3, New York-London: Acad. Press, 1957, pp. 68o-684
Scientific version:	17.Mar.8o
Text version:	28.Sep.81

2501091344

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

SDS_polyacrylamide_gel_electrophoresis

Principle:	separation of negatively charged complexes of proteins with a detergent (SDS) acccording to their relative molecular mass in an electrical field across a vertical polyacrylamide gel (1o o/o) with defined pore size and a discontinuous buffer system
Time	
Sampling:	on days ..
Determination:	within ... days after sampling
Sample material and quantity:	protein SDS complexes 5 to 3o ul equiv. to 5 to 3o ug protein/slot
Results expressed in:	-
Equipment:	magnetic stirrer: Ika Combimag RCO, Janke und Kunkel GmbH, D-7813 Staufen
	pH meter: PW 94o9, glass electrode: CA/14o2, Philips GmbH, D-35oo Kassel
	syringes: Hamilton, 1o or 5o ul, Parafilm, via Hirtz und Co., D-5ooo Köln 5o
	glass tubes: length: 12o mm inner diameter: 4 mm
	glass cell: Desaga Doppeltrennzelle, thickness: 1.5 mm width: 22o mm length: 11o mm volume: 37 ml
	electrophoresis apparatus: system Havana, Desaga GmbH, D-69oo Heidelberg
	power supply: no. 21o3, LKB Instruments, D-8o32 Gräfelfing

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Chemicals and reagents:

acrylamide, no. A 8887,
N,N'-methylene-bis-acrylamide (Bis),
no. M 7256,
trizma base (Tris), no. T 15o3,
N,N,N',N'tetramethylethylenediamine
(TEMED), no. T 8133,
glycine, no. G 7126,
Sigma Chemie GmbH,
D-8o21 Taufkirchen

ammonium persulfate, no. 13375,
sodium dodecyl sulfate (SDS), no.
2o76o,
Serva Feinbiochemica GmbH,
D-69oo Heidelberg 1

sucrose, no. 7654,
E.Merck,
D-61oo Darmstadt 1

gel composition:

acrylamide	1o	o/o
Bis	o.15	o/o
ammonium persulfate	o.o25	o/o
TEMED	o.oo2	o/o
buffer	o.375	mol/l

electrode buffer composition:

Tris	25	mmol/l
glycine	o.2	mol/l
SDS	o.1	o/o
final pH (a)	8.3	

Procedure:

glass cells or tubes placed in buffer,
o.o25 mol Tris, o.192 mol glycine,
o.1 o/o SDS, pH 8.3 at 9 degrees cen-
tigrade

samples applied with Hamilton syringe
current: 94 mA for 2 glass cells or
1 mA/tube for cylindrical gels
starting voltage: approx. 9o V

tracking dye velocity: approx. 2 cm/h

Scientific version: 7.Jan.81
Text version: 25.Jun.81

(a) in absence of SDS

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Serum preparation

Principle:	after clotting separation of serum from blood clot by centrifugation
Time	
Sampling:	-
Determination:	on day of sampling
Sample material and quantity:	blood, approx. 1.2 ml
Results expressed in:	-
Equipment:	centrifuge: type "Eppendorf", no. 5412, micro vials: type "Eppendorf", no. 381o, polypropylene, Netheler und Hinz, D-2ooo Hamburg 63
Chemicals and reagents:	-
Procedure:	blood samples kept in closed micro vials at RT for approx. 3o min.
Centrifugation:	9.8x1oE4 m/s2 (= 995o x g) for 2 min at RT. Transfer of serum supernatant to fresh micro vial
Storage:	at o to 4 degrees centigrade
Scientific version:	1.Sep.8o
Text version:	8.Oct.81

2501091347

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Determination_of_excised_skin_area

Principle:	planimetric determination of area of excised skin spread on graph paper
Time:	(a)
Sample material and quantity:	excised skin area, 1 piece/mouse
Results expressed in:	mm2
Equipment:	digitizer: MOP-AM o3, Kontron Messgeräte, D-8o57 Eching
Chemicals and reagents:	-
Procedure:	drawing of skin areal contours on graph paper planimetric determination of area with digitizer equipment (a)
Scientific version:	15.Apr.81
Text version:	25.Jun.81

(a) Details will be given in the report

2501091348

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Silanization of glass surfaces

Principle:	covalent binding of silane derivation to hydroxyl groups of glass surface in order to prevent electrostatic cell adherence
Time	
Sammpling:	-
Determination:	-
Sample material and quantity:	pasteur pipettes or other glass ware
Results expressed in:	-
Equipment:	-
Chemicals and reagents:	dichloro-dimethylsilane (DCDS), no. 803452, Merck-Schuchardt, D-8011 Hohenbrunn
Procedure:	immersion of glass in DCDS solution (1 ml/l DCDS in acetone), for 10 s, glass then rinsed 5 times with distilled water and dried in hot air (50 degrees centigrade) for 1 h
Scientific version:	22.Aug.79
Text version:	29.Sep.81

2501091349

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Determination of sodium concentration

Principle:	flame photometric determination of the intensity of the characteristic sodium emission in relation to the emission of an internal lithium standard
Time	
Sampling:	-
Determination:	within .. .. after sampling
Sample material and quantity:	(1) urine, 24 ul (2) perfusate supernatant, 24 ul (3) serum, 24 ul
Results expressed in:	mmol/l
Equipment:	KLiNa-flame photometer, no. 65271o, Beckman Instruments GmbH, D-8ooo München 4o
Chemicals and reagents:	internal lithium-standard: 15 mmol/l, no. 663229 sodium/potassium standard: sodium: 14o mmol/l, potassium: 5 mmol/l, no. 663223, Beckman Instruments GmbH, D-8ooo München 4o
Procedure:	according to manufacturer's recommendations
Scientific version:	6.Mar.79
Text version:	24.Sep.81

2501091350

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Staining of proteins in polyacrylamide gels with Coomassie blue

Principle:	binding of stain to fixed proteins, removal of excess dye by diffusion
Time:	fixation immediately after electrophoresis to prevent diffusion of protein
Sample material and quantity:	-
Results expressed in:	-
Equipment:	diffusion destainer for round gels: Bio-Rad Laboratories GmbH, D-8ooo München 5o diffusion destainer for flat gels: Desaga GmbH, D-69oo Heidelberg 1
Chemicals and reagents:	propanol-2, no. 9634, acetic acid, no. 62E, Coomassie brilliant blue R25o, no. 12553, E. Merck, D-61oo Darmstadt 1
Procedure:	fixation: at least 1 h in circulating fixation solution: 25o ml/l propanol-2, 1oo ml/l acetic acid staining: up to 1 h in fixation solution plus 125 g/l filtered Coomassie brilliant blue
Scientific version:	13.Oct.8o
Text version:	25.Jun.81

2501091351

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Storage of clones

Principle:	freezing of cells for unlimited preservation
Time:	-
Sample material and quantity:	1 to 5x1oE6 cells/(ml x tube)
Results expressed in:	-
Equipment:	cell freezer controller and liquid nitrogen refrigerator, Union Carbide, Durham, United Kingdom Nunc Kryo-tubes 2 ml, no. N 1o76-1/3 634o1, Nunc GmbH, D-62oo Wiesbaden
Chemical and reagents:	dimethyl sulfoxide (DMSO), no. 9678, E. Merck, D-61oo Darmstadt 1 fetal calf serum, no. 629, Difco Laboratories, Detroit, Michigan, USA
Procedure:	cells collected, centrifuged with 9.8x1oE3 m/s2 (= 1ooo x g) for 1o min and resuspended in a mixture containing 9oo ml/l fetal calf serum and 1oo ml/l DMSO, frozen down in programmed cell freezer and stored in liquid nitrogen
Scientific version:	2o.Nov.8o
Text version:	8.Oct.81

2501091352

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Surgical Preparation

Equipment:	cotton thread, 1oo o/o cotton, Amann 2ooo
	PVC tubing: inner diameter: 2 mm outer diameter: 3 mm with catheter tip
	PVC catheter, Braunüle type o, sterile and pyrogen-free, Braun Melsungen AG, D-35o8 Melsungen
	syringes: 5 ml, plastic, sterile and pyrogen-free, Pharma-Plast GmbH, D-33oo Braunschweig
Chemicals and reagents:	urethane administration solution: 233 g/l bidistilled water, urethane, no. 849o, E. Merck, D-61oo Darmstadt 1
	heparin administration solution: 1oo U/ml 9 g/l sodium chloride solution, heparin, no. 2459o, Serva Feinbiochemica GmbH und Co., D-69oo Heidelberg 1

Procedure

Anesthesia:	intraperitoneal administration of 1.18 g urethane/kg BW equivalent to 13.3 mmol/kg BW
Nerve and tendon preparation:	blunt preparation of the Nervus ischiadicus on each HL and loose clasping with cotton thread. Preparation of both Tendi m. tricipitis surae (Achilles), fixation of cotton thread in tendon, separation of tendons from Tuber calcanei
Blood vessel operation:	tight ligatures at the following blood vessels (venae and arteriae simultaneously) or organs in the given order (see also FIGURE .):

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A. epigastrica inferior
A. circumflexa ilium profunda
A. iliaca interna
A. sacralis mediana
A. mesenterica inferior
A. mesenterica superior
Urinary bladder and prostate
A. renalis
A. testicularis
A. gastrica sinistra
A. hepatica
Aa. lumbales
A. inliaca lumbales

loose ligature around A. abdominalis and Vena cava inferior caudally to A. renalis

tight ligature around A. abdominalis and Vena cava inferior cervically to A. renalis (= start of circulation standstill)

insertion of PVC tube into incision of V. cava inferior, fixation of tube with caudal ligature

insertion of PVC catheter into incision of A. abdominalis, fixation of catheter with caudal ligature

Heparinization: instillation of approx. 2o ml heparin solution with syringe through PVC catheter into HL.

Killing: perforation of the diaphragm immediately after instillation of heparin solution

Isolation of HE from supper part of body: fixation of steel bar perpendicular to body axis at the lower kidney poles

Scientific version: 3o.Jan.8o
Text version: 19.Aug.81

2501091354

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Determination of temperature

Principle:	resistance thermometer (Pt 1oo) reading
Time:	.. min after start of perfusion
Sample material and quantity:	perfusate, sites of determination: (see FIGURE ..) (1) reservoir (2) venous tubing
Results expressed in:	degrees centigrade
Equipment:	digital thermoelement: R4o/2, Messgeräte-Werk Lauda, Dr. R. Wobser KG, D-697o Lauda-Königshofen digital thermometer: H11, Hugo Sachs Elektronik KG, D-78o1 March-Hugstetten/Freiburg
Chemicals and reagents:	-
Procedure:	platinum probe (Pt 1oo) completely submersed in perfusate stream directly behind the perfused organ and in reservoir
Scientific version: Text version:	3.Dec.79 7.Oct.81

250109135

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Thin-layer chromatography

Principle:	chromatographic separation based on differential adsorption to and/or distribution of substances in a mobile phase and stationary cellulose thin-layer phase
Time:	within 2 h after preparation of test or reference substance solution
Sample material and quantity:	test or reference substances dissolved in bidistilled water, 1 to 1o ul equiv. to 1o to 1oo ug/plate
Results expressed in:	Rf (a)

Equipment

Chromatography:	thin-layer plates: cellulose F layer on glass plate, no. 57o8, E. Merck, D-61oo Darmstadt 1 spray gun: C. Desaga GmbH, D-69oo Heidelberg filter paper: no. 2524o, UV-lamp, no. 292oo, Camag, D-1ooo Berlin
Photography:	MP4 Land camera, Land Polacolor 2 film, type 668, Polaroid GmbH, D-6ooo Frankfurt/Main-Niederrad 1
Other equipment:	analytical balance: model 2oo1 MP2, Sartorius GmbH, D-34oo Göttingen

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(a) quotient of test or reference substance migration from point of application and distance of mobile phase front migration

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Chemicals and reagents:

bromophenol blue, no. 8122,
butanol, no. 199o,
methanol, no. 6oo7,
ammonia, no. 5426,
formic acid, no. 264,
acetic acid, no. 62
dioxane, no. 9671,
E. Merck,
D-61oo Darmstadt 1

benzol, no. 8o14,
chloroform, no. 7152,
Baker Chemikalien,
D-6o8o Gross-Gerau

ethanol, denaturated,
Bundesmonopolverwaltung für Branntwein,
D-5ooo Köln 1

Procedure

Thin-layer chromatography: according to Stahl: Dünnschicht-Chromatographie, Berlin, Heidelberg: Springer Verlag (1967), S. 293

mobile phase (solvent phase mixtures: see TABLE .)

Photography: reflected white light

Scientific version: 24.Oct.8o
Text version: 19.Jul.81

2501091357

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Preparation of tissue homogenate supernatant = S9 fraction

Principle:	mechanical grinding and centrifugation with 8.8x1oE4 m/s2 (= 9ooo x g) of tissue
Time	
Sampling:	on day ...
Determination:	within ... days after sampling
Sample material and quantity:	organs, ... pooled/group
Results expressed in:	-
Equipment:	homogenizer: Potter-Elvehjem, glass, with motor-driven teflon pestle, Braun Melsungen AG, D-35o8 Melsungen Multifix, model M 8o, Alfred Schwinher, D-7o7o Schwäbisch-Gmünd centrifuge: RC-5B, rotor: SS-34, Du Pont Instruments, D-635o Bad Nauheim freezer: no. 8218, Forma Scientific, Marietta, Ohio, USA tubes: polycarbonate, no. 3137, polypropylene, no. 3138, Du Pont Instruments, D-635o Bad Nauheim polypropylene, no. 381o, Netheler und Hinz, D-2ooo Hamburg 63
Chemicals and reagents:	potassium chloride, no. 4936, E. Merck, D-61oo Darmstadt 1

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Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Procedure	according to Ames B.N., McCann, J. and Yamasaki, E., Mutation Research 31: 347-364 (1975)
Killing:	stretching of spine, followed by 3o s exsanguination from inferior vena cava and abdominal aorta
	short submersion of organs in 15o mmol/l potassium chloride, pH 7.o, determination of wet weight, freezing of whole organ in liquid nitrogen
Storage:	in jars at minus 8o degrees centigrade
Homogenization:	after addition of 3 volumes of 15o mmol/l potassium chloride, pH 7.o, to the organ wet weight (1 g equiv. to 1 ml) mechanical grinding in Potter-Elvehjem apparatus with motor-driven teflon pestle, approx. 2oo rpm, 4 degrees centigrade
	glassware and equipment in contact with homogenate: precooled
Centrifugation:	8.8x1oE4 m/s2 (= 9ooo x g), for 1o min, 4 degrees centigrade
Storage of S9 fraction:	minus 8o degrees centigrade, 1 ml aliquots

Scientific version: 7.Dec.8o
Text version: 12.Jul.81

2501091359

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Determination of triglyceride concentration

Principle:	photometric determination of the NADH oxidation during the formation of lactate from pyruvate, which is produced from phosphoenolpyruvate by pyruvate kinase (EC 2.7.1.4o). ADP necessary for this reaction is formed from ATP and glycerol (glycerokinase EC 2.7.1.3o). Glycerol is obtained from triglycerides after hydrolysis by lipase (EC 3.1.1.3) and esterase (EC 3.1.1.1)
Time	
Sampling:	on day ..
Determination:	on day of sampling
Sample material und quantity:	serum, 5 ul
Results expressed in:	mmol/l
Equipment:	ABA-1oo Bichromatic Analyzer, Abbott Laboratories, Diagnostic Division, So. Pasadena, California 91o3o, USA
Chemicals and reagents:	test set Triglycerides, UV method, no. 126 o12, Boehringer Mannheim GmbH, D-68oo Mannheim 31
Procedure:	according to Wahlefeld, A.W. in: Bergmeyer, H.U. (Ed.): Methoden der enzymatischen Analyse, 3rd ed., Vol. 2, Verlag Chemie Weinheim, 1974, p. 1878
	photometric determination:
	wavelength 1: 34o nm
	wavelength 2: 38o nm
Scientific version:	1.Sep.78
Text version:	28.Sep.81

2501091360

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Determination of urea concentration

Principle:	photometric determination of NADH oxidation during the reaction of alpha ketoglutarate with ammonia (glutamate dehydrogenase, EC 1.4.1.2), formed from serum urea by urease (EC 3.5.1.5)
Time	
Sampling:	-
Determination:	-
Sample material and quantity:	serum, 2.5 ul
Results expressed in:	mmol/l
Equipment:	ABA-1oo Bichromatic Analzyer, Abbott Laboratories, Diagnostics Division, So. Pasadena, California 91o3o, USA
Chemicals and reagents:	test set BUN A. Gent, no. 6oo7, Abbott Laboratories, Diagnostics Division, So. Pasadena, California 91o3o, USA
Procedure:	according to Talke, H., Schubert, G.E., Klin. Wochenschr. 43: 174 (1965), with modifications by Abbott Diagn. photometric determination: wavelength 1: 38o nm wavelength 2: 34o nm
Scientific version:	1.Sep.78
Text version:	23.Sep.81

2501091361

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000

Determination of wet weight

Principle:	gravimetric determination
Time	
Sampling:	on day ..
Determination:	immediately after dissection
Sample material and quantity:	rat organs, 5 organs pooled
Results expressed in:	g
Equipment:	scales: no. 1212 MP, Sartorius GmbH, D-34oo Göttingen
Chemicals and reagents:	-
Procedure:	-
Scientific version:	21.Sep.8o
Text version:	9.Oct.81

2501091362

Source: https://www.industrydocuments.ucsf.edu/docs/mghl0000